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The Molecularly Imprinted Polymers. Influence of Monomers on The Properties of Polymers - A Review

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***Abstract*- The synthesis of MIPs for two types of templates (herbicides, and flavonoids) and their application in analytical chemistry are discussed. Particular attention has been paid the issue of bonding the template and selection of appropriate monomer in different types of compounds. This short review aims at presenting the molecular imprinting technology (MIT) which is considered as an attractive method to produce impressive receptors for application in analytical chemistry. The challenge of designing and synthesizing a molecularly imprinted polymer (MIP) can be a daunting prospect to the uninitiated practitioner, simply because of the number of experimental variables involved, e.g. the nature and levels of template, functional monomers, cross-linkers, solvents, initiators and even the method of initiation and the duration of polymerization. Indubitably, the most important place of the polymer is its "heart" or the cavity corresponding to the template and the way it is attached to the molecule of the template.**

***Index Terms:* molecularly imprinted polymer, adsorption capacity, functional monomers, herbicides, flavonoids**

I. INTRODUCTION

In recent years, molecular imprinting technology (MIT) has become an attractive method to produce impressive receptors [1]-[2]. This technology provides smart polymers which have recognition sites complementary to a target molecule called a template [3]. The technology for obtaining MIPs is based on the formation of a complex between an analyte (template) and a functional monomer in a selected solvent that acts as a porogen. The complex is then polymerized trough thermal or photo-initiation in the presence of a cross-linker and a three-dimensional polymer network is formed. The template is removed from the polymer structure leading to the formation of specific binding sites [4]. Molecularly imprinted polymers (MIPs) show high affinity to and specificity for the particular target molecule. The removal of the template molecules leaves in MIPs the recognition cavities that are complementary to the template molecules in terms of shape, size and location of functional groups (Fig.1).

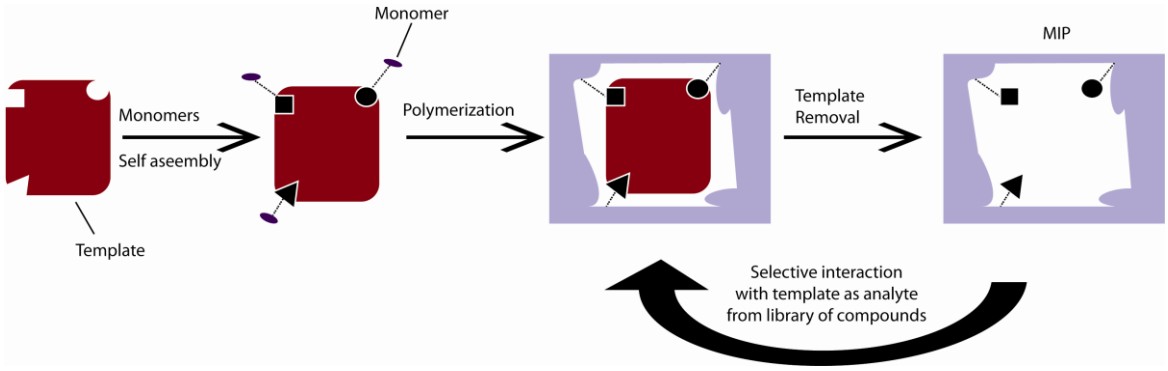

Fig.1. Schematic representation of the molecular imprinting process

The main parameters determining the properties of the polymers obtained are: the type of monomers non-covalently interacting with the template, the relation of monomers/template concentrations, the method and conditions of polymerization, the method of template removal from the polymer. The artificial particles, in contrast to the natural antibodies, show many advantages, such as high chemical stability, excellent reusability, relatively easy, reproducible and low cost synthesis [5]. Therefore, MIPs heave been widely used in many areas such as chromatographic separation, membrane separations,
chemical sensors and biosensors, catalysts and solid-phase extraction [3]-[4],[6]-[7]. The latter application has recently enjoyed greater interest, because molecular imprinting coupling solid-phase extraction has become the frequently used technique in enrichment and extraction of trace amounts of target analytes in samples with complex composition [8]-[10]. MIPs are currently used in many analytical chemistry fields, for example: for the recognition of antibiotics in food and environmental samples [11], in pharmaceutical applications, drug discovery, drug purification or drug delivery [3], for analysis of auxins in plant tissues [12], determination of polycyclic aromatic hydrocarbons in several kinds of samples water or cigarettes

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[13]. MIPs show extensive application potential in separation science, food analysis, and biological and environment analysis and in other fields. MIPs are endowed with two of the most important features of biological receptors - the ability to recognize and bind to specific target molecules. However, MIPs in contrast to biological receptors, are large, rigid, and insoluble, whereas their natural counterparts are smaller, flexible, and, in most instances, soluble.

However, on the other hand MIPs also have drawbacks such as slow mass transfer, irregular shape, incomplete template removal, poor site accessibility or heterogeneous distribution of binding sites. MIP can be isolated from water solutions by filtration or centrifugation. These processes create difficulties for small size of polymers and strong interaction of the polymer with highly ionic water solution. Much effort has been undertaken to solve the above issues. One of the promising alternatives is imparting magnetism to MIPs and then using magnetic separation. In addition, magnetic support can be easily isolated from the real samples using a magnet. Magnetic molecularly imprinted polymers (mag-MIPs) are endowed with high affinity to templates as the traditional MIPs. Mag-MIPs allow monitoring targeted compounds in complex environmental matrices in a fast, cheap and accurate way [14]. If some magnetic components are encapsulated into MIPs, the resulting composite polymers, magnetic MIPS (mag-MIPs) not only heave magnetically susceptible characteristics, but also have selectivity to the guest molecules [15]. The mag-MIPs are prepared by encapsulating inorganic magnetic particle with an organic polymer, which results in a product that combines the advantages of high recognition properties of MIP and the handling convenience of magnetic separation. Meanwhile, the magnetic separation process can be performed directly in crude samples, which is especially useful for large-scale operation. Therefore, mag-MIPs could be promising multifunctional candidates for the adsorption and separation process [6],[10],[16]-[18]. Such polymers can be separated easily and rapidly in the preparation and synthesis process without wasting any materials and time to perform the separation by centrifugation or filtration process [3]. Mag-MIPs have been synthesized by different methods, such as suspension polymerization, emulsion polymerization, surface imprinting polymerization and others [7]. Magnetic Fe_3O_4 nano or microparticles are the commonly used magnetic components. Nevertheless, the limited specific surface area of magnetic particles and low number of functional groups may result in low density of recognition sites. Therefore, the efforts to improve the imprinting efficiency of mag-MIPs are continued. The most commonly used preparation process includes the following four steps. The first step is the preparation of magnetic nanoparticles, such as Fe_3O_4. The second step is the surface modification or functionalization of the magnetic components. The third step is surface-imprinted polymerization using functionalized nanoparticle as a magnetic core in the presence of the template molecule, functional monomer and cross-linker. The fourth step is to remove the template molecules from the polymer [11],[15]. The general steps of the preparation of magnetic molecularly imprinted polymer (mag-MIP) presented Fig. 2. The MIPs process on Fe_3O_4 modified surface is analogous to that of polymer synthesis.

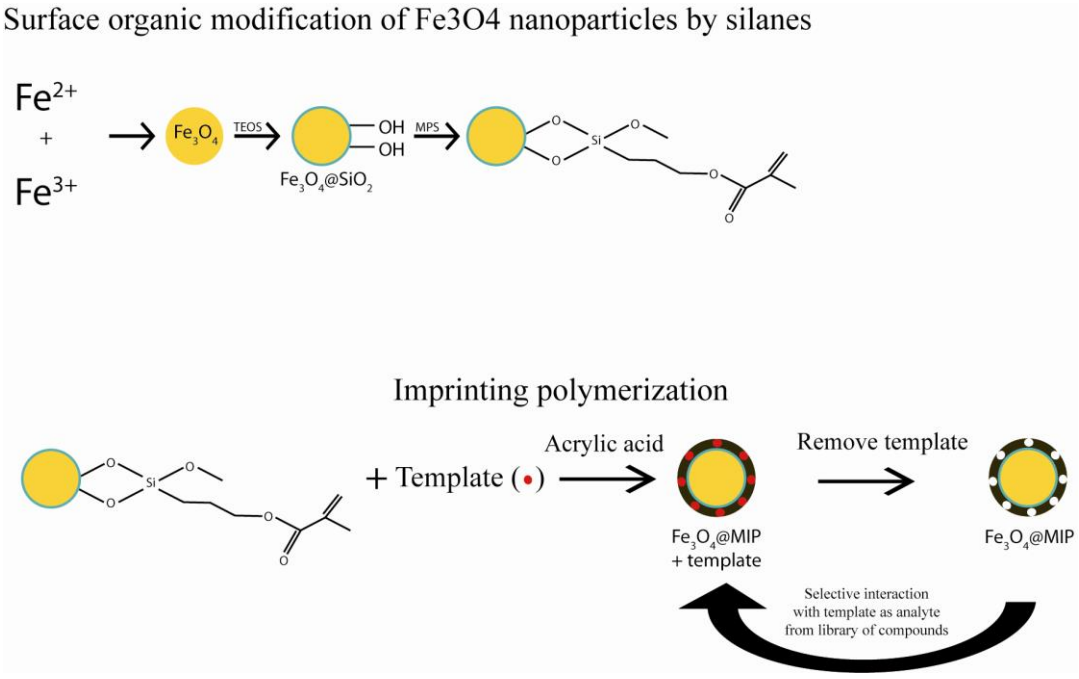
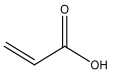
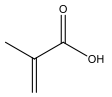
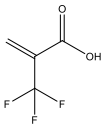
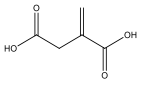
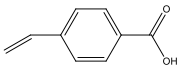
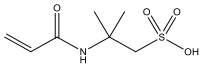
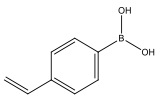
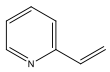
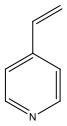
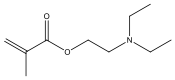
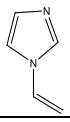
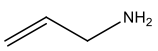
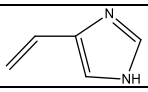


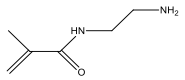
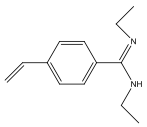
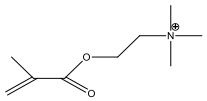
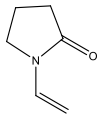
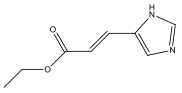
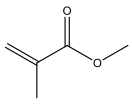
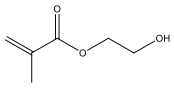
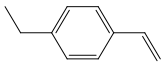
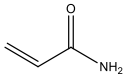
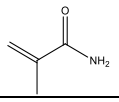
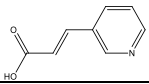
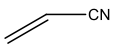
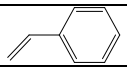
Fig. 2. The general steps of the preparation of magnetic molecularly imprinted polymer (mag-MIP)

In any case, regardless of whether we deal with MIPs or mag-MIPs, the most important site of the polymer is its "heart" that determines the way it attaches to the template molecule. In order to synthesize a molecularly imprinted polymer (MIP) selective to one analyte, it is important to choose the nature and levels of templates, functional monomers, cross-linkers, solvents, initiators of polymerization and even the method of initiation and the

duration of polymerization. The master molecule is dissolved in a selected solvent called a porogen, together with functional monomers capable of polymerization (Table 1).

Table 1. Functional monomers used in MIPs synthesis

Character	Structural pattern	Name
Acidic		acrylic acid (AA)
		methacrylic acid (MAA)
		2-(trifluoromethyl)acrylic acid (TFMAA)
		itaconic acid
		p-vinylbenzoic acid
		2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA)
		4-vinylbenzeneboronic acid
Basic		2-vinylpyridine (2-VP)
		4- vinylpyridine (4-VP)
		N,N-(diethylaminoethy methacrylate) (DEAEM)
		1-vinylimidazolo
		allylamine
		4-(5)-vinylimidazole

		N-(2-aminethyl)-methacrylamide
		N,N'-diethyl-4-styrylamidine
		N,N,N-trimethyl aminoethylmethacrylate
		N-vinylpyrrolidone (NVP)
		urocanic etyl ester
Neutral		methyl methacrilate (MMA)
		2-hydroxyethyl methacrylate (2-HEMA)
		4-ethylstyrene
		acrylamide
		methacrylamide
		trans-3-(3-pirydyl)-acrylic acid
		acrylonitrile
		styrene

MIPs can interact non-covalently (electrostatic interactions, hydrophobic interactions, hydrogen bonds), or make covalent bonds between the template and the monomers. Upon addition of the cross-linking agent (Table 2) and the proper polymerization reaction, a highly crosslinked polymer structure is formed. The chemical structures of selected chemical initiators are shown in Table 3.

Table 2. The cross-linkers used in MIPs synthesis

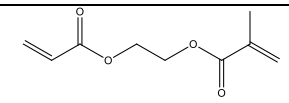
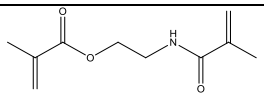
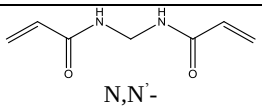
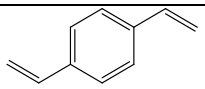
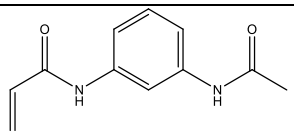
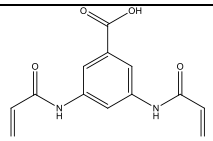
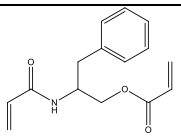
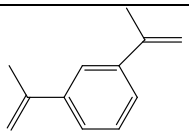
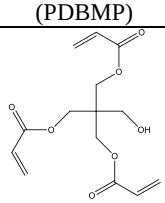
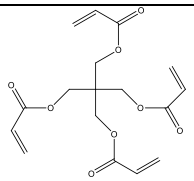
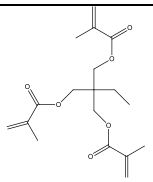
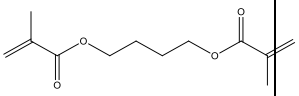
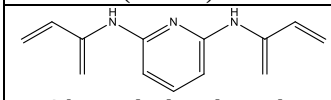
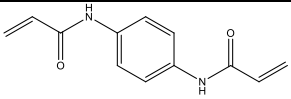
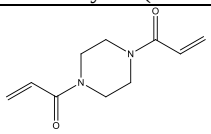
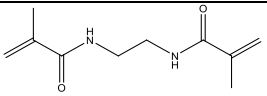
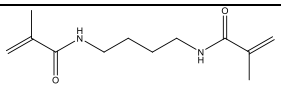
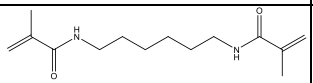
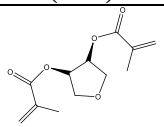
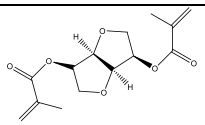
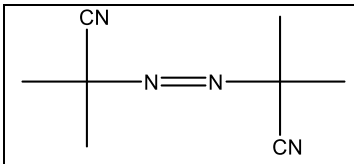
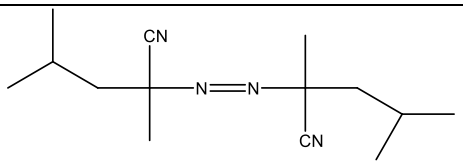
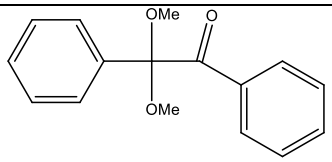
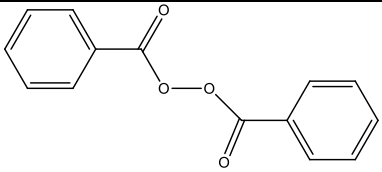
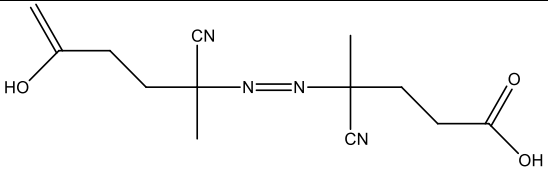
 ethylene glycol dimethacrylate (EGDMA)	 N,O-bismethacryloyl ethanolamine	 N,N'-methylenebisacrylamide (MDAA)	 p-divinylbenzene (DVB)
 N,N'-1,3-phenylenebis(2-methyl-2-propenamide) (PDBMP)	 3,5-bisacryloylamido benzoic acid	 N,O-bisacryloyl-L-phenylalaninol	 1,3-diisopropenyl benzene (DIP)
 pentaerythritol triacrylate (PETRA)	 pentaerythritol pentacrylate (PRTEA)	 triethylolpropane trimethacrylate (TRIM)	 tetramethylene dimethacrylate (TDMA)
 2,6-bisacryloylamidopyridine	 1,4-phenylene diacrylamide	 1,4-diacryloyl piperazine (DAP)	 N,N'-ethylene bismethacrylamide
 N,N'-tetramethylene bismethacrylamide	 N,N'-hexamethylene bismethacrylamide	 anhydroerythritol dimethacrylate	 1,4;3,6-dianhydro-D-sorbitol-2,5-dimethacrylate

Table 3. The chemical structures of selected chemical initiators

 azobisisobutyronitrile (AIBN)	 azobisdimethylvaleronitrile (ABDV)	 dimethylacetal of benzil
 benzoylperoxide (BPO)	 4,4'-azo(4-cyanovaleric acid)	

The most important stage is the selection of functional groups of the monomer to match the functional groups present in the template molecule, and further allow for creation of a permanent prepolymerization complex and build imprinting sites in the polymer imprinting process. If two or more types of functional monomers are used in polymerization reaction of MIPs should be account for the reactivity of both monomers. The relative reactivities of many monomers have been defined and tabulated, and are presented in the form reactivity ratio for given monomer pairs. The reactivity ratio assumes values from 0 to 1. If functional monomers have similar reactivities, their contribution to the polymer chain formation is similar. If monomers differ significantly in reactivity, the monomer of higher reactivity brings a greater contribution to the polymer chains formation. Many functional monomers

showing different physicochemical properties are available as commercially available chemical products. The ones containing atypical groups can be synthesized in laboratory. The monomers are usually divided into: acidic, basic and neutral. Undoubtedly, the most popular acid monomer is methacrylic acid (MAA), while the most popular basic one is 2- or 4-vinylpyridine (2-VP or 4-VP). The use of various neutral monomers in polymerization resulted in ineffective imprinting. The best results have been obtained for acrylamide or its N-alkyl derivatives, especially those using less polar solvents during polymerization.

In this review the syntheses of MIPs and mag-MIPs with the use of two types of templates: herbicides and flavonoids, and their application in analytical chemistry, are discussed. Particular attention has been paid to the issue of bonding of the template and selection of the appropriate monomer.

II. Characterization Of Templates

II.I. Herbicides

In recent years, the hazards of using pesticides have been accentuated because of the fears augmented by the sharp rise in their use in agriculture and industry. Herbicides, known also the weed killers, are chemical substances used to control unwanted plants. Selective herbicides control specific weed species, whilst leaving the desired crop relatively unharmed. On the other hand, the non-selective herbicides (sometimes called "total weed killers") can be used to clear waste ground, industrial and construction sites, railways and railway embankments as they kill all plant material with which they come in contact. Some of them act by interfering with the growth of the weed and their operation employs plant hormones. Some plants produce natural herbicides, such as the genus Juglans - walnuts. Herbicides are widely used in agriculture and in landscape turf management. They are applied in total vegetation control (TVC) programs for maintenance of highways and railroads. Smaller quantities are used in forestry, pasture systems, and management of areas set aside as wildlife habitat. Herbicides have been alleged to cause a variety of health effects ranging from skin rashes to death. The pathway of attack can arise from improper application resulting in direct contact with field workers, inhalation of aerial sprays, food consumption and from contact with residual soil contamination. Herbicides can also be transported via surface runoff to contaminate distant surface waters which is another pathway of their ingestion. Some herbicides decompose rapidly in soil and other types have more persistent characteristics with longer environmental half-lives. The issue is illustrated by the example of two popular herbicides.

a) Atrazine – (2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine)

Atrazine is a white colored solid organic compound. It is a member of the triazine class compounds which are basically herbicides used for controlling the broad leaf weeds in crops such as sugarcane, corn, sorghum, pine, grapes, and in residential lawns, roadway grasses and forestry products. It belongs to the Restricted Use Pesticide (RUP) category, implying that only registered professionals can apply it, while its use is prohibited for the general public. It became more popular due to its effectiveness against a large spectrum of weeds. Atrazine is generally found in ground water, drinking water and surface water and is highly persistent. Atrazine has toxic nature in waste water and is an environmental threat which affects the ecosystem and human health as it is related to immune-suppression, reproductive abnormalities, cancer and hormone disruption. According to the World Health Organization (WHO), the maximum contaminant level (MCL) of atrazine in drinking water is 0.2 ppb. The hazards related to atrazine use and the strict environmental regulations make the sensing of atrazine an important problem.

b) 2,4-D – (2,4-dichlorophenoxyacetic acid)

Phenoxy herbicides are currently among the most frequently used pesticides worldwide. They have been used on a large scale in agriculture to control the growth of broad-leaved weeds on rice, maize, wheat, and in post-emergence applications in most developing countries. Among them, 2,4-D is a common important phenoxy herbicide that is selective, systemic auxin-type herbicide extensively used throughout the world for the past 50 years. The compound 2,4-dichlorophenoxyacetic acid is odorless, white to tan solid and sinks in water. 2,4-D is a chief component of commonly used herbicides and has been frequently detected in water. It is harmful to human beings and threatens our health. In June 2015 the World Health Organization's (WHO) International Agency for Research on Cancer (IARC) confirmed its 1987 classification of 2,4-D as a possible carcinogen. EPA reviewed 2,4-D as part of the Six Year Review and determined that the 70 ppb MCL for 2,4-D are still protective of human health [19].

II.II. Flavonoids

Flavonoids comprising more than 4000 species are a large family of plant secondary metabolites widely distributed in the plant kingdom in a broad range of commonly consumed fruits and vegetables and plant-derived products such as cocoa, tea or wine. Flavonoids comprising the subclasses of flavonols, flavones, catechins (flavanols), flavanones, anthocyanidins and isoflavonoids have biological effects in many mammalian cell systems, in vitro as well as in vivo. Flavonoids have been reported as responsible for several beneficial health effects due to their strong antioxidant capacity, such as antimutagenic, antidiabetic, anti-inflammatory qualities and prevention against several kinds of cancer. Although their pharmaceutical properties have been widely studied, the research into their purity and selectivity is still in progress. Although catechins are widely used natural antioxidants, the concentration of these antioxidants usually found in natural matrices has been reported as high only in tea samples. Therefore, and because of the complexity of natural matrices, the use of catechins from these samples needs sample enrichment and purification. Owing to its previously reported high selectivity, affinity and simplicity, SPE involving a molecular imprinted polymer (MISPE) has been applied as a selective sorbent material for the cleanup and preconcentration of several target compounds from

biological and environmental samples. The flavonol quercetin is a typical member of the large family of flavonoids.

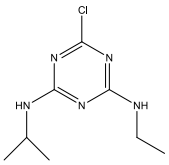
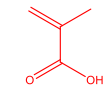
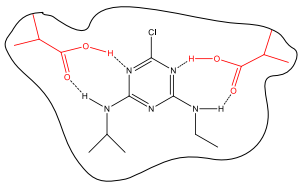
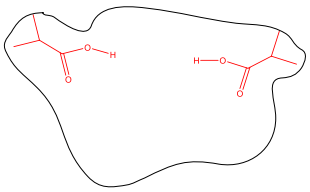
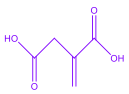
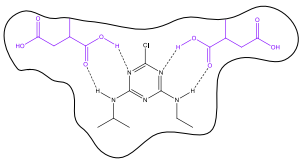
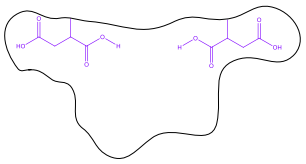
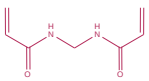
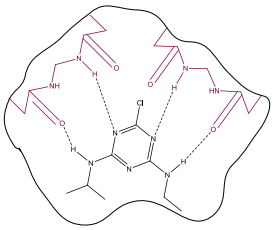
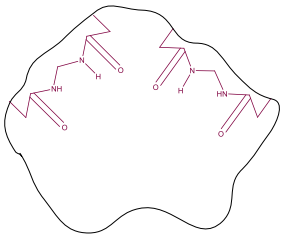
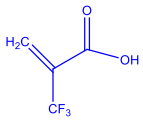
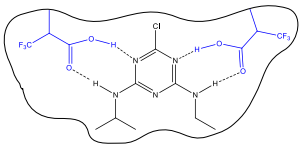
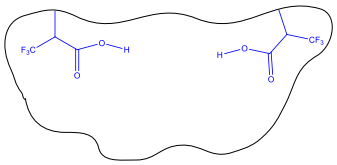
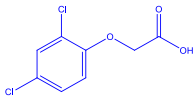
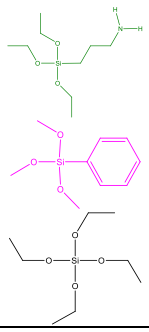
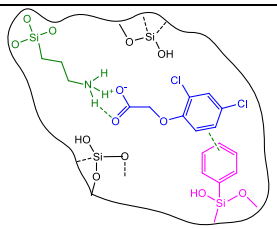
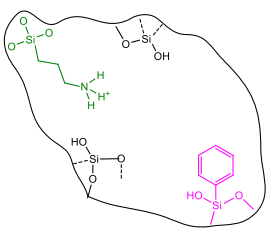
a)Quercetin - (3,3,4,5,7-penta-hydroxy flavone)

Quercetin is the most active antioxidant of the flavonol family. The richest sources of quercetin are capers, followed by onions, asparagus, lettuce and berries; in many other fruit and vegetables it is present in smaller amounts, between 0.1 and 5 mg/100 g. Besides antioxidant properties, quercetin shows antitumor and antiviral properties as well as aids in functioning of the immune system. Chromatographic methods have been widely used for the isolation of quercetin from plants, employing a variety of solvent combinations. Because of the existence of quercetin in nature at low concentrations and its structural similarity to other flavones in natural states, its separation is very difficult [20].

III. Characterization Of MIPs

The synthesis of MIPs for two types of templates (herbicides, and flavonoids) and their application in analytical chemistry has been discussed. Table 4 presents the monomers used for the syntheses of imprinted polymer nanoparticles showing high selectivity to certain herbicides and flavonoids.

Table 4. Structure of MIPs obtained with different monomers

Templat	Monomer	Imprinted polymer nanoparticle	Non-imprinted polymer nanoparticle- cavity	Ref.
a) Atrazine 				[19]-[28]
				[24]-[25]
				[24]-[25]
				[25]
b) 2,4-D 				[29]
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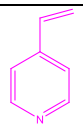
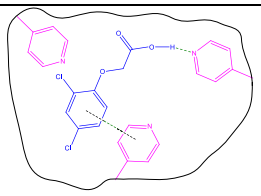
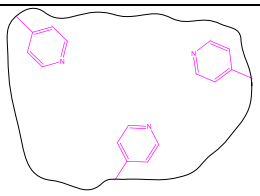
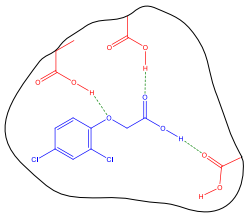
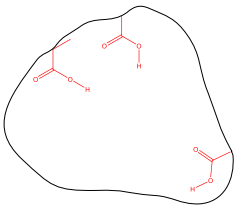
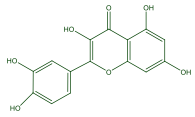
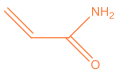
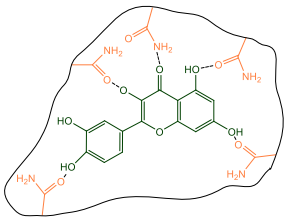
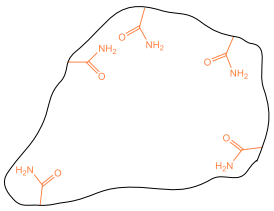
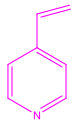
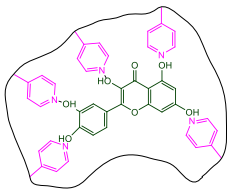
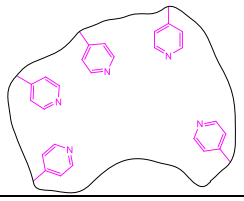
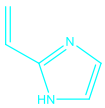
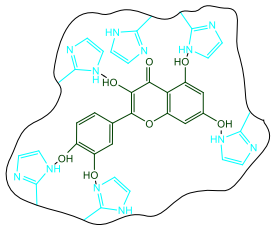
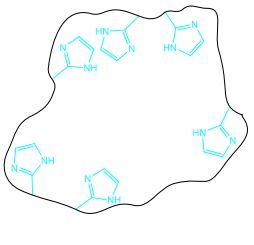
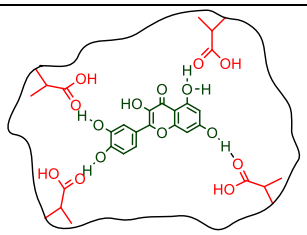
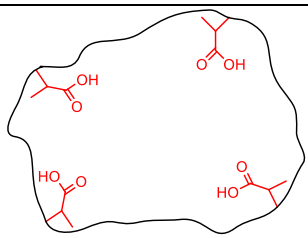
				
				[33]
c) Quercetin 				[34]-[41]
				[35]-[37] [42]-[45]
				[46]
				[36]-[38] [42] [47]-[48]

Four types of molecularly imprinted polymers containing atrazine template were analyzed. Different monomers were used to synthesize these molecules, thus creating specific cavities selective to the molecule of the template. For this purpose the following monomers were used: methacrylic acid, itaconic acid (IA), N,N'-methylene-bis-acrylamide and 2-(trifluoromethyl)acrylic acid (TFMAA). All MIPs with all monomer and at all monomer/template ratios considered showed some imprinting effects and selectivity for the template. The best performance was shown by MAA at 2:1 (monomer/template ratio) and bisacrylamid at 3:1, with both polymers also showing excellent selectivity much better than their structural analogues. However, the most favorable ratio of monomer/template is 4:1 for most polymers. Furthermore MAA MIPs had a higher binding capacity and specificity toward the template molecule than IA MIPs. This can be attributed to the fact that in the MAA type of nanoparticles the carboxyl groups of two MAA molecules are associated via non-covalent interactions with the secondary amino groups of a single atrazine molecule. On the other hand, in IA nanoparticles the active site is formed via the association of the two carboxyl groups of a single IA molecule with the template molecule. Moreover, both polymers have a higher binding efficiency and specificity toward the template molecule than the TFMAA MIPs, even though TFMAA is a more acidic and, thus, a

better hydrogen bond donor than MAA. This can be explained by the negative inductive effect of the three fluoro substituents of TFMAA resulting in stronger interactions with the ester functionalities of EGDMA, which lower the specific binding capacity of MIPs toward the atrazine molecule. It is worth noting, however, that not only the monomer is important, but the monomer/template ratio, because the 3:1 bisacrylamide based MIPs gave practically the same performance as the 2:1 MAA polymer, a result that clearly indicates the value of the modeling approach, as bisacrylamide is usually only considered as a cross-linker rather than a functional monomer. Generally, the aims of sample pretreatment are twofold: reduction or elimination of interferences and concentration of analytes. Various modern sample pretreatment methods, such as solid phase extraction (SPE), solid phase microextraction (SPME), supercritical fluid extraction (SFE) and continuous flow liquid membrane extraction (CFLME), have been exploited for extracting pesticide residues from various farm products, but not only. However, these techniques also have their drawbacks. SPME, columns are usually brittle and vulnerable, showed low sensitivity and poor stability in some actual cases. The SFE apparatus is usually huge, complex and expensive, which limits its use. The process of CFLME is not easy to control, which makes its application difficult. In the last decade, SPE has aroused great interests by virtue of short analysis time, high recovery and enrichment factor, and less reagent consumption. Micro-extraction by packed sorbent (MEPS) is a miniaturized form of SPE technique, which has become an attractive and powerful sample preparation approach. It is well known that adsorbents are the key factors influencing the efficiency of SPE. It is obvious that the more selective the SPE sorbents are, the higher sensitivities of the analyses can be achieved. The recognition mechanism of MIPs was attributed to three-dimensional cavities that were complementary to the template molecule or structural analogues in shape, size and chemical functionality. The characteristic properties of MIPs, such as good mechanical and chemical stability, low cost, good selectivity, high adsorption capacity, and favorable reusability, make molecularly imprinting technology more accessible to analysis. The routine analytical methods for the determinations of triazine extracts form farm products include high performance liquid chromatography (HPLC), gas chromatography, capillary electrophoresis, gas chromatography–tandem mass spectrometry (GC–MS/MS), and liquid chromatography–tandem mass spectrometry (LC–MS/MS). It has been proved that the selectivity of the absorbent employed in the sample pretreatment for enrichment and purification of triazine herbicides significantly affects the performance of the analytical methods. This topic has been showed on the basis of on triazines but this solution has been used in analytical chemistry by many chemical groups [18]-[30].

Three types of molecularly imprinted polymers containing 2,4-D template were analyzed. Three different monomers were used to synthesize these molecules, thus creating specific cavities selective to the template molecules. These monomers were methyl methacrylate, 4-vinylpyridine (4-VP) and aminopropyltrimethoxysilane (APTES), tetraethoxysilane (TEOS), phenyltrimethoxysilane (PTMOS). In the latter example PTMOS interacts with the template 2,4-D trough hydrophobicity and π-π stacking, but APTES interacts with this template trough electrostatic interaction and hydrogen bonds. It was confirmed using the FT-IR spectra presented by Yanli Sun's. PTMOS was also found to play a more important role than PTMOS in the process of MIPs formation. The best concentration and selectivity coefficient values were found at the molar ratio of 20:1.5:1 (TEOS:APTES:PTMOS). Equally important is the amount of template used and the type of monomers used. At low concentration of 2,4-D, the adsorption capacity of MIPs increased with increasing amount of 2,4-D. When the molar ratio of the monomers to the template reached 6.25, the MIPs showed the maximum adsorption capacity. It has been proved that the fewer the template molecules, the smaller the porosity of MIPs. In the presence of a large number of template molecules , the structure of MIPs becomes too loose to be able to maintain a tree-dimensional structure of the cavities. On the basis of the analysis performed, the most suitable polymer was chosen. The adsorption capacity increased at first rapidly and then it remained constant after a contact time of 3h. Adsorption equilibrium was reached in a relatively short period of time, indicating the stronger force and less mass transfer resistance between MIPs and template. The maximum adsorption capacity of MIPs toward 2,4-D appeared at pH about 7. The adsorption of 2,4-D on MIPs obeyed the Langmuir model. The MIP prepared in the optimum conditions were characterized by large adsorption capacity and high selectivity. 4-VP has been also proposed as a template. The FT-IR spectra show the formation of intermolecular H-bonding between the carboxylic group of 2,4-D and the nitrogen of 4-VP. It has been found that the molar relationships between the template and functional monomer influence the quantity and quality of MIP recognition sites. Hence 2,4-D imprinted and non-imprinted polymers were prepared at 1:2 and 1:4 template-monomer ratio. The 1:4 systems offered high specific binding when compared to that of the 1:2 systems. To evaluate the variation of rebinding with concentration of 2,4-D solution, batch methods were applied using a stock solution of 2,4-D with concentration ranging from 0.3 to 1.5 mM. The extent of 2,4-D binding increased with concentration up to 0.9 mM and then remained constant. To optimize the time taken to reach the maximum binding of 2,4-D by MIP and NIP, defined amounts of the polymer were equilibrated with template solution of a known concentration and the binding was followed spectrophotometrically at certain time intervals. The MIP attained saturation within 4-6 h. Selectivity studies with structural analogues revealed that, besides the complementarity in size and shape, the optimal spatial fit also affected the binding interactions. Another functional monomer used was methacrylic acid. The preferred molar ratio of the template: functional monomer was 1: 4 for this compound. Thermogravimetric analysis plot for MIP can prove the thermal stability of the synthesized polymer. The adsorption by MIP was approximately constant from pH 2 to 4, but decreased from pH 5 to 9. Therefore, pH 4 was chosen as optimized pH, for quantitative adsorption of 2,4-D on the synthesized MIPs in

the subsequent experiments. The equilibrium sorption time was established in experiment. Consequently, the optimum equilibration time of 10 minutes was obtained for quantitative removal of 2,4-D from solution into the solid phase [31]-[35].

Four types of molecularly imprinted polymers containing Quercetin template were analyzed. Different monomers had been used to synthesize them, thus specific cavities selective to the molecule of the template were created.. For this purpose the following monomers were used: acrylamide, 4-VP, vinyl imidazole (VI) and methacrylic acid. In most of the present research, the imprinted particles are obtained from bulk polymerization by non-covalent approach. Up to now, successful imprints have been made with affinity to various classes of compounds. However, it is generally believed that compounds soluble in polar solvents are not recommended as templates because polar solvents can disturb the non-covalent cohesions between the templates and functional monomers. Quercetin molecules are strongly polar and include no hydrophobic functional groups which can be dissolved in THF at the usual imprinting concentration. Acrylamide was chosen as the functional monomer because it is favorable for methacrylic acid associating with templates by hydrogen-bonding interaction in polar environment. Consequently the compounds soluble in polar solvents still could be imprinted by non-covalent method in polar solvents as long as the negative action caused by polar solvents was not so strong to be complexation impeding [36]. The quercetin molecule contains five hydroxyl groups and one carbonyl group which can form hydrogen bonds with functional groups such as hydroxyl, amino and carbonyl groups. From the structure of quercetin both γ-OH and ε-OH of the quercetin molecule can form strong intramolecular hydrogen bonds with the 1-carbonyl group, and therefore the inter-molecular interaction between the template and monomer is weakened. The remaining three hydroxyl groups as the main imprinting functional groups supply strong intermolecular hydrogen bonds with the polymer and control the precise imprinting sites. Five apparent selective binding sites are in the quercetin molecule. After removal of the template molecules the specific imprinting sites are maintained. These sites selectively adsorb quercetin molecules, when AA is used. The molar ratio monomer/template should be higher than 4: 1. At low monomer content it is difficult to create stable printing sites. However, in both studies [41],[42] the scholars argue that the highest quercetin adsorption and the highest the polymerization efficiency is achieved by using 4-VP as monomer. Theoretically, 4-VP is a suitable monomer because Brønsted basic functional monomers e.g., 2- or 4-vinylpyridine (VP), diethylaminoethylmethacrylate (DEAEMA) are preferably chosen for templates containing acidic groups, whereas acidic functional monomers e.g., MAA, trifluoromethylacrylic acid (TFM), itaconic acid (IA) are used to target Brønsted bases. Porogenic solvents play an important role in the formation of the porous structure of MIPs, known as macroporous polymers. Therefore in the non-covalent imprinting technique, the presence of polar solvents, such as water, disrupts the interaction of the template with the monomer, which results in polymers with poor level of recognition. The nature and level of porogenic solvents determine the strength of non-covalent interactions and influence polymer morphology, which directly affects the performance of MIP. In addition, the proper choice of porogenic solvent was crucial, due to the low solubility of flavonoids. The polarity and hydrophobicity of flavonoid compounds are very diverse and vary depending on the side groups. Quercetin is a strongly polar molecule with no hydrophobic functional groups, and can be dissolved in THF and EDMA at the cross-linking monomer. The choice of functional monomer is crucial as it maintains the stability of template-monomer complexes during the imprinting processes, in stabilizing the binding site and affinity. 4-VP was selected as functional monomer because of the H-bond accepting property of the pyridine nitrogen. Such a functional moiety of 4-VP is complementary to quercetin, which contained 5H-donor sites from hydroxyl groups. Previously, systemic investigation of the influence of monomer and crosslinking monomer on the properties of MIPs for quercetin has been carried out [39, 41-45]. MAA or acrylic acid (AA) has been commonly used as functional monomers in making MIPs. However, MAA or AA show strong nonspecific interaction with the imprinted molecule and the hydrogen bonds formed between MAA or AA and the imprinted molecule are destroyed in a polar solvent such as acetonitrile. Xu et al. have used AA as a functional monomer and TRIM as a cross-linker to prepare quercetin-imprinted polymer, the polymer has obvious selectivity and could partly separate quercetin from isorhamnetin. For separation of flavonoid compounds by molecularly imprinting technique, besides MAA, also AA and acrylamide were used as functional monomer; 4-vinyl pyridine was usually used as a functional monomer to imprint flavonoids because of its weak basicity. A. Molinelli et al.[46] have also used 4-vinyl pyridine as the functional monomer to selective determination of quercetin and selective recognition for the isolation of quercetin from wine samples has been achieved. This MIPs showed excellent selectivity toward quercetin and was therefore suitable for the application in solid-phase extraction (SPE). It has been acknowledged that quercetin is a polyphenol and the phenolic hydroxyl groups behave as weak acid, so the phenolic hydroxyl groups can form strong hydrogen-bonding interaction with the pyridyl group. Recently, chitosan used as an adsorbent has drawn much attention because of its high contents of amino and hydroxyl functional groups showing high potentials of the adsorption of dye, metal ions, and proteins. Other useful features of chitosan include its abundance, nontoxicity, hydrophilicity, and biocompatibility. Therefore, to improve the selectivity of MIP, polymerization using MAA as the functional monomer and chitosan beads as functional matrix was employed in this work. The chitosan beads act not only as the skeleton supporting matrix to give the MIP beads spherical shape, but as functional groups supporters to afford amino groups [37]-[50].

Sample preparation is an important step in most analytical processes. The samples are treated prior to their analysis to remove interferents from matrices and to improve the selectivity of the analytical method. The development of new sorbent materials aims at enhancement of selectivity,

adsorption capacity, simplicity, robustness, resistance in a wide range of pH, temperatures and solvents, and physical–mechanical stability at low cost. Several sorbents can be used in SPE, however, in recent years, MIPs have been demonstrated as promising sorbents in SPE and have been applied in chromatographic stationary phases, chiral separations, antibody mimics, drug delivery systems and many others. According to the principles of green chemistry, the miniaturization of sample preparation techniques has been highlighted due to its low consumption of samples, solvents and reagents, by using MIPs these assumptions can be fulfilled [23],[51].

IV. CONCLUSIONS

The most common approach towards preparation of MIPs for SPE applications is based on non-covalent imprinting, which facilitates rapid uptake and release of the analyte. Covalent imprinting may not be suitable for template separations because these systems show kinetically controlled binding involving the formation of stable covalent bonds. The polymerization conditions (type and concentration of monomers, cross-linking level, temperature, solvent) are important variables determining MIPs preparation. Moreover, the optimum composition of a MIP can depend on the solvent in which is to be used (and thus on the sample matrix in some cases). To obtain MIPs with the best recognition properties, combinatorial synthesis accompanied by high-throughput screening, and molecular modelling approaches have been developed. The latter uses molecular modelling software to screen a virtual library of monomers for a given template. Monomers that form the most stable complex with the template are identified, and the corresponding MIPs are synthesized for experimental confirmation. The combinatorial MIP optimization is based on the preparation of a library of different MIPs, from which the best one is selected for strong and selective target binding and low non-specific binding.

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